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Application and verification of simultaneous determination of cellulose $\delta^{13}C$ and $\delta^{18}O$ in *Picea shrenkiana* tree rings from northwestern China using the high-temperature pyrolysis method

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Abstract: Stable isotopes in tree-ring cellulose provide important data in ecological, archaeological, and paleoenvironmental researches, thereby, the demand for stable isotope analyses is increasing rapidly. Simultaneous measurement of cellulose $\delta^{13}C$ and $\delta^{18}O$ values from tree rings would reduce the cost of isotopic commodities and improve the analytical efficiency compared with conventional separate measurement. In this study, we compared the $\delta^{13}C$ and $\delta^{18}O$ values of tree-ring α-cellulose from Tianshan spruce (*Picea schrenkiana*) in an arid site in the drainage basin of the Urumqi River in Xinjiang of northwestern China based on separate and simultaneous measurements, using the combustion method (at 1050°C) and the high-temperature pyrolysis method (at 1350°C and 1400°C). We verified the results of simultaneous measurement using the outputs from separate measurement and found that both methods (separate and simultaneous) produced similar $\delta^{13}C$ values. The two-point calibrated method improved the results (range and variation) of $\delta^{13}C$ and $\delta^{18}O$ values. The mean values, standard deviations, and trends of the tree-ring $\delta^{13}C$ obtained by the combustion method were similar to those by the pyrolysis method followed by two-point calibration. The simultaneously measured $\delta^{18}O$ from the pyrolysis method at 1400°C had a nearly constant offset with that the pyrolysis method at 1350°C due to isotopic-dependence on the reaction temperature. However, they showed similar variations in the time series. The climate responses inferred from simultaneously and separately measured $\delta^{13}C$ and $\delta^{18}O$ did not differ between the two methods. The tree-ring $\delta^{13}C$ and $\delta^{18}O$ values were negatively correlated with standardized precipitation evapotranspiration index from May to August. In addition, the $\delta^{18}O$ was significantly correlated with temperature (positive), precipitation (negative), and relative humidity (negative) from May to August. The tree-ring $\delta^{13}C$ and $\delta^{18}O$ values determined simultaneously through the high-temperature pyrolysis method could produce acceptable and reliable stable isotope series. The simultaneous isotopic measurement can greatly reduce the cost and time requirement compared with the separate isotopic measurement. These results are consistent with the previous studies at humid sites, suggesting that the simultaneous determination of $\delta^{13}C$ and $\delta^{18}O$ in tree-ring α-cellulose can be used in wide regions.

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1 Introduction

Tree-ring stable isotopes provide important data for analyses in dendroclimatology, dendroecology, and paleoecology (Schleser et al., 1999; McCarroll and Loader, 2004; Babst et al., 2014). The measurement and interpretation of stable isotopes in tree-ring cellulose are increasingly influential and are rapidly improving the development of dendroclimatology, dendroecology, and paleoecology (McCarroll and Loader, 2004; Dawson and Siegwolf, 2007; Werner et al., 2012). These studies have greatly improved our understanding of the past environmental variation (Treydte et al., 2007; Leavitt et al., 2010; Liu et al., 2017) and of the plant responses to the current environment or the changing climate (Saurer et al., 2014; Altieri et al., 2015; Frank et al., 2015). Recently, stable isotopes records in tree rings have been incorporated into isotope-enabled Earth system models to evaluate the large-scale mechanisms of variation in photosynthesis and stomatal conductance under a changing climate and environment (Saurer et al., 2014; Keel et al., 2016; Keller et al., 2017). However, the veracity of the implementation of these models needs to be confirmed by a closer examination of regional and long-term isotopic chronologies in tree rings.

The stable carbon isotope (δ^{13}C) and stable oxygen isotope (δ^{18}O) are traditionally measured separately. Unfortunately, high cost of sample preparation coupled with time-consuming analytical procedures are required to obtain a high level of replication and an adequate spatial coverage which are required for robust paleoclimatology studies and are difficult to achieve (Leavitt et al., 2010). Obtaining stable isotope measurements based on a long-term chronology is difficult due to the limited quantity of wood available in narrow tree rings from arid regions (Wang et al., 2013) and from archaeological studies (such as subfossil wood) (Kłusek and Pawełczyk, 2014). Furthermore, when trends in two or more stable isotopes are viewed together, this can provide far greater insights into plant physiology and environmental responses than the stable isotopes that are considered separately (Loader et al., 2015). For example, the relationship between δ^{13}C and δ^{18}O reflects a balance between plant carbon and water metabolism (Babst et al., 2014; Liu et al., 2014). Tree-ring δ^{18}O mainly reflects the δ^{18}O of the source water and environmental humidity, whereas tree-ring δ^{13}C reflects the variations in photosynthetic rate and stomatal conductance (Farquhar et al., 1982; Roden et al., 2000; Roden and Farquhar, 2012). Therefore, the combination of δ^{13}C and δ^{18}O could capture more environmental signals simultaneously than a single isotope, and could improve our understanding of the plant's physical response to climate change and of the terrestrial carbon cycle in relation to climate change (Scheidegger et al., 2000; Babst et al., 2014). Thus, it would be beneficial to develop rapid and high-efficiency methods for a reliable stable isotope analysis by combining the two stable isotopes (i.e., δ^{13}C and δ^{18}O).

To accomplish this goal, some researchers investigated the potential for simultaneous measurement of δ^{13}C and δ^{18}O from low- or high-temperature pyrolysis of cellulose (Knöller et al., 2005; Young et al., 2011; Woodley et al., 2012). The time and cost of such an approach would decrease substantially, since multiple isotopic ratios could be analyzed in the same time, without an extra expense. Several comparative studies have reported encouraging results with this approach (Saurer et al., 1995; Knöller et al., 2005; Young et al., 2011; Woodley et al., 2012). However, these studies were conducted in humid areas (Knöller et al., 2005; Young et al., 2011; Woodley et al., 2012). Few researches have been conducted in arid areas, where the range of δ^{18}O value is relative small (Xu, 2014).

The purposes of the present study were to confirm the applicability of simultaneous measurement of calibrated $\delta^{13}C$ and $\delta^{18}O$ in tree-ring α-cellulose from Tianshan spruce (*Picea schrenkiana*) in climatic and ecological studies and to determine whether it is possible to compare stable isotope values obtained from this method with values from separate measurement in a previous research conducted in an arid site in Xinjiang of northwestern China. First, we determined the relationships between the reaction temperature and the simultaneously measured $\delta^{13}C$ and $\delta^{18}O$ values to provide a reference for the comparison of stable isotope estimates based on the pyrolysis method at different reaction temperatures. In this analysis, we confirmed the viability and stability of the pyrolysis method for the simultaneous measurement of $\delta^{13}C$ and $\delta^{18}O$ from tree-ring α-cellulose. Next, using previous data from separate measurements of $\delta^{13}C$ (combustion analysis) and $\delta^{18}O$ (pyrolysis at 1350°C) in tree-ring α-cellulose from *P. schrenkiana*, we compared the values of $\delta^{13}C$ and $\delta^{18}O$ from separate measurement with those from simultaneous measurement by the high-temperature (at 1400°C) pyrolysis of tree-ring α-cellulose. Finally, we evaluated whether the $\delta^{13}C$ and $\delta^{18}O$ values measured simultaneously by the high-temperature pyrolysis of tree-ring α-cellulose could be used for paleoenvironmental and paleoecological researches in arid regions.

2 Data and methods

2.1 Materials and experimental design

We obtained tree-ring samples from *P. schrenkiana* growing in an arid site in the drainage basin of the Urumqi River in Xinjiang Uygur Autonomous Region of northwestern China. The tree-ring cores were obtained from mature trees using a 12-mm-diameter increment borer at breast height (about 1.3 m above the ground) in September 2008. We totally collected 40 cores from 20 mature trees (two cores per tree) and processed these cores according to procedures described by Stokes and Smiley (1968). The tree-ring width was measured and cross-dated using a Lintab device (LINTAB 6; Rinntech, Heidelberg, Germany) connecting with the Time Series Analysis and Presentation dendrochronological software. We selected 10 of these cores at a rate of one core per tree and pooled the wood materials for a given year to extract α-cellulose based on the method of Loader et al. (1997). The α-cellulose was extracted and then homogenized using an ultrasound machine (JY92-2D, Ningbo Scientz Biotechnology Company, Ningbo City, China) (Laumer et al., 2009). The composite samples were first used for the separate analyses in our previous study (Xu et al., 2014) and the remaining samples were used for the simultaneous analyses in this study.

The $\delta^{13}C$ was measured in 2010 using the conventional method of combustion at 1050°C in an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific, Milan, Italy) coupled with a continuous-flow isotope ratio mass spectrometer (Delta Plus, Thermo Electron Corporation, Bremen, Germany) at the Key Laboratory of Western China's Environmental System, Ministry of Education, Lanzhou University, China. In the combustion analysis, the tin capsules packaged 0.18–0.20 mg α-cellulose were transformed into CO_2 in the presence of excess oxygen (Young et al., 2011). The analytical uncertainty was 0.05‰ (=1σ) for $\delta^{13}C$. For $\delta^{18}O$ measurement, we loaded 0.14–0.16 mg of α-cellulose into silver capsules and determined the isotope ratio using a high-temperature conversion elemental analyzer (TC/EA; Thermo Electron Corporation, Bremen, Germany) at 1350°C coupled to a Finnigan MAT-253 mass spectrometer (Thermo Electron Corporation, Bremen, Germany) at the State Key Laboratory of Cryospheric Science, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences (CAS), Lanzhou, China. Hereafter, we refer to this $\delta^{18}O$ chronology as the "pyrolysis-1350" series. More details of the sample preparation and stable isotope measurements were provided in Xu et al. (2014).

The simultaneous measurement of $\delta^{13}C$ and $\delta^{18}O$ was conducted using a TC/EA coupled to a Finnigan MAT-253 mass spectrometer at the State Key Laboratory of Cryospheric Science, Northwest Institute of Eco-Environment and Resources, CAS. Specifically, we used a laboratory standard material (Sigma-Aldrich α-cellulose; Chemical Abstracts Service No. 9004-34-6, Germany) to test the effects of measurement conditions on simultaneous measurement of both isotopes. To determine the most suitable reaction temperature for the pyrolysis, we tested the results with reaction temperatures ranging from 1000°C to 1440°C. We used a ceramic outer tube, a glassy-carbon inner tube, and a graphite electrode during the pyrolysis. We packed 0.16–0.18 mg Sigma-Aldrich α-cellulose into a silver capsule. High-purity helium (99.9%) was used as the carrier gas, at a pressure of 1.2 bar (equivalent to 17 psi). Every sample was run for 800 s. The pyrolysis products (CO and H_2) were separated using a gas chromatograph under 65°C.

We defined the optimal temperature of approximately 1400°C for the pyrolysis reaction because the temperature produced the smallest deviation from the known isotopic values in the laboratory standard material. We heated the samples in a vacuum drying oven at 65°C for 48 h before measurements. We then loaded 0.16–0.18 mg α-cellulose of tree rings into a silver capsule. Afterwards, the packaged samples were heated again in a vacuum drying oven at 65°C for 24 h before the analysis to ensure the samples were dry. For the simultaneous measurement of stable isotopes, we set temperatures of 1400°C for the furnace and of 65°C for the gas chromatograph. Each tree-ring α-cellulose sample was tested twice. The analytical precision of the standards was lower than 0.21‰ for $\delta^{18}O$ and 0.10‰ for $\delta^{13}C$. We tested the tree-ring α-cellulose sample again if the standard deviation of the two measurements was higher than 0.3‰ for $\delta^{18}O$ and 0.15‰ for $\delta^{13}C$. All of the stable isotope results were represented using delta (δ) notation and were expressed as "‰" values relative to the international standard Vienna Pee Dee Belemnite (VPDB) for the carbon isotope and to the Vienna Standard Mean Ocean Water (VSMOW) for the oxygen isotope. Hereafter, we refer to these results as the "pyrolysis-1400" series.

2.2 Correction of mass spectrometer analysis and $\delta^{13}C$ corrections

We calibrated the stable isotope measurements using two methods: the multi-standard calibration and the two-point calibration (Evans et al., 2016). The runtime drift correction, amplitude correction, and bias correction in mean and deviation were conducted in the two-point calibration (Evans et al., 2016). In the multi-standard calibration, we compared the stable isotope values with the mean values from four standards with known values. Here, we used the Sigma-Aldrich α-cellulose standard and three IAEA (International Atomic Energy Agency) standards (IAEA-CH3 standard, benzoic acid IAEA 601, and IAEA-C3 standard) for the calibration (Table 1). In the two-point calibration, we used two standard cellulose materials (Sigma-Aldrich α-cellulose and IAEA-CH3; Table 1), and performed the correction based on the mean difference between the measured values and the mean of these two known standard values.

Table 1 Statics of standard materials during α-cellulose calibration

Reference material	$\delta^{13}C$		$\delta^{18}O$	
	Value (‰)	Standard deviation (‰)	Value (‰)	Standard deviation (‰)
IAEA-CH3	–24.72	0.05	32.52[b]	0.25
Benzoic acid IAEA 601	–27.50[a]	0.15	23.30	0.30
IAEA-C3	–24.91	0.49	32.61[b]	0.40
Sigma-Aldrich α-cellulose	–24.25[a]	0.10	28.20[b]	0.20

Note: All of the stable isotopes are from the IAEA (International Atomic Energy Agency) products except for the values with a superscript letter. All of the work to calibrate the reference materials was conducted by at least two laboratories. [a], the value was calibrated with USGS24 ($\delta^{13}C$= –16.05‰ (±0.04‰)) and USGS40 ($\delta^{13}C$= –26.39‰ (±0.09‰)). [b], the value was calibrated from the benzoic acid IAEA 601.

For analysis of long-term trends in the $\delta^{13}C$, it is necessary to correct for the effect of isotopically depleted CO_2 in the atmosphere as a consequence of industrialization (Feng and Epstein, 1996; McCarroll and Loader, 2004). In addition, tree-ring $\delta^{13}C$ series may incorporate an additional trend that reflects a change in the tree's physiological response to increasing atmospheric CO_2 concentrations (McCarroll et al., 2009; Gagen et al., 2011; Frank et al., 2015). In this study, we used the "pin" (pre-industrial) correction method to correct the $\delta^{13}C$ series ($\delta^{13}C_{pin}$) for the effect of the increasing atmospheric CO_2 concentrations. The correction was implemented in the MATLAB software (McCarroll et al., 2009; McCarroll, 2010). This offered a conservative approach for dealing with the CO_2 effect and has been successfully applied in tree-ring ecological researches (Andreu-Hayles et al., 2011) and dendroclimatological studies (Hafner et al., 2011; McCarroll et al., 2011; Xu et al., 2014; Lavergne et al., 2017). We used the $\delta^{13}C_{pin}$ to detect the climatic signals recorded in tree-ring $\delta^{13}C$.

2.3 Climate data

We used all available climate data (during 1951–2008) from a low-elevation station in Urumqi (43°46′48″N, 87°37′12″E; 919 m a.s.l.) located about 70 km northeast of the tree-ring sampling site, to explore the climate responses of tree-ring $\delta^{13}C$ and $\delta^{18}O$ based on combustion and pyrolysis methods. These climate records were obtained from the Chinese Meteorological Data Center (http://data.cma.cn/), and were also used in our previous study (Xu et al., 2014). The homogeneities of these data had been confirmed. The climate variables included mean monthly temperature, total monthly precipitation, and mean monthly relative humidity. We also used mean monthly values of the standardized precipitation evapotranspiration index (SPEI) (Vicente-Serrano et al., 2010) from the grid point (43°N, 87°E) nearest the sampling site to detect the responses of tree-ring $\delta^{13}C$ and $\delta^{18}O$ series to drought.

2.4 Statistical methods

We used independent-sample *t*-test to detect the difference in the mean for $\delta^{13}C$ values determined by combustion and pyrolysis methods, and for $\delta^{18}O$ values from the pyrolysis method at 1350°C and the simultaneous measurement (pyrolysis at 1400°C). We used one-way analysis of variance (ANOVA) to detect differences in $\delta^{13}C$ and $\delta^{18}O$ values between the two methods. We applied linear regressions to explore the trends in the $\delta^{13}C$ and $\delta^{18}O$ series from 1950 to 2008. To determine whether the time series produced by the two methods differ from each other, we compared the trends in these series by examining the slope of the regressions.

We calculated the value of Pearson's correlation coefficient (r) for the relationships between the tree-ring isotope data ($\delta^{13}C_{pin}$ and $\delta^{18}O$) and the climate variables (mean monthly temperature, total monthly precipitation, monthly relative humidity, and SPEI) during the common period from 1951 to 2008. To compare these results with previously published findings (Xu et al., 2014), we performed this analysis for the period from September of the previous year (the year before the tree-ring formed) to September of the current year.

3 Results and discussion

3.1 Dependence of α-cellulose $\delta^{13}C$ and $\delta^{18}O$ on the pyrolysis temperature

Uncalibrated measured values of tree-ring $\delta^{13}C$ and $\delta^{18}O$ for the Sigma-Aldrich α-cellulose from simultaneous measurement both increased with rising furnace temperature, particularly at temperatures above 1250°C (Fig. 1). The mean measured $\delta^{18}O$ showed a small difference (2.1‰) for the Sigma-Aldrich α-cellulose standard at temperatures from 1000°C to 1250°C. The mean measured $\delta^{13}C$ also showed a small difference (0.74‰) among the same temperature ranges. The standard deviation of the measurement was relatively large for $\delta^{18}O$ (0.31‰–0.73‰) and the number of outliers was relatively high when the furnace temperature was lower than 1250°C (Fig. 1). These results indicate a relatively low stability of the measurement at temperatures lower than 1250°C using the TC/EA because the α-cellulose was only partially (about 5/6) converted into CO at these temperatures (Leuenberger and Filot, 2007; Young et al., 2011).

At temperatures from 1300°C to 1440°C, the mean measured $\delta^{18}O$ (without calibration) showed a large difference (4.20‰) for the Sigma-Aldrich α-cellulose. The difference between the mean measured values was 0.90‰ for $\delta^{18}O$ and 0.16‰ for $\delta^{13}C$ at temperatures of 1300°C–1350°C (Fig. 1). The difference decreased to 0.49‰ for $\delta^{18}O$ but increased to 0.27‰ for $\delta^{13}C$ at temperatures of 1400°C–1440°C, which are nearly the acceptable precisions for $\delta^{18}O$ (0.30‰) and $\delta^{13}C$ (0.20‰). The standard deviation was 0.25‰ for $\delta^{18}O$ and 0.10‰ for $\delta^{13}C$ at the temperature of 1400°C (Fig. 1). Thus, high-temperature (1400°C) pyrolysis of tree-ring α-cellulose resulted in a nearly complete conversion of oxygen into CO (Leuenberger and Filot, 2007; Woodley et al., 2012). Measurement at 1400°C was more stable and precise than measurement at lower temperatures, and was able to prolong the lifetime of the furnace compared to higher temperatures. In comparing temperature ranges, previous studies have found that the reaction temperature of 1400°C appears to be optimal for the simultaneous measurement of α-cellulose δ^{18}O and δ^{13}C (e.g., Woodley et al., 2012).

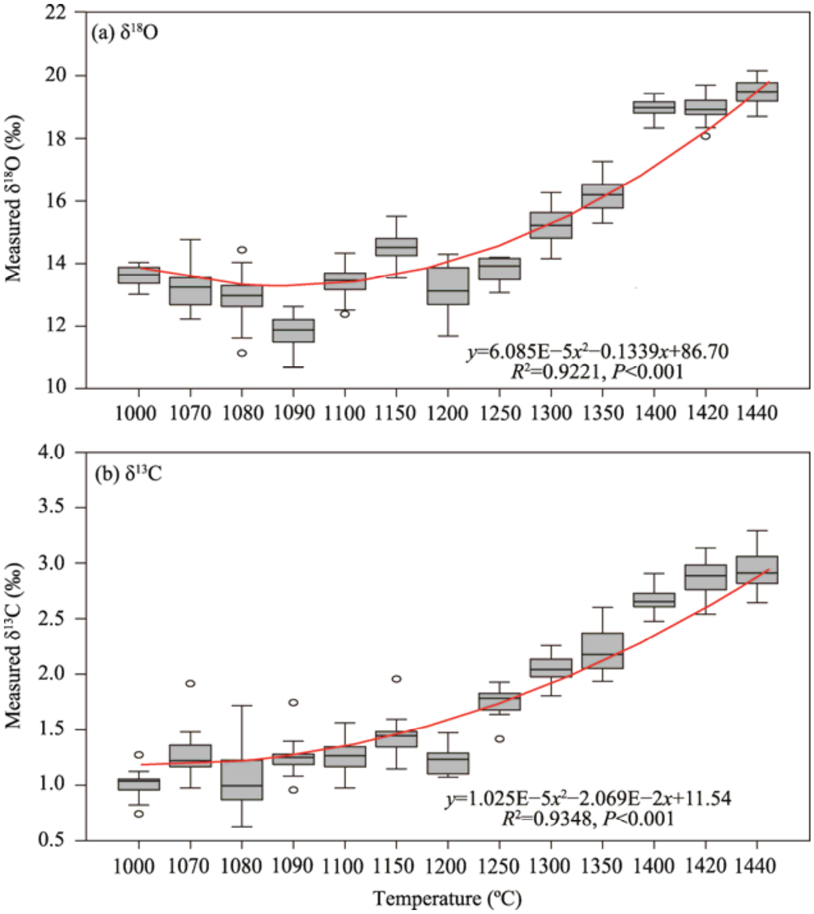

Fig. 1 Temperature-dependence of the simultaneously measured (a) δ^{18}O and (b) δ^{13}C from the Sigma-Aldrich α-cellulose. The box plots represent the measured values, median (horizontal line), minimum and maximum values (whiskers), and the 25th to 75th percentiles. The open dots represent outliers (values more than 2 SD (standard deviation) from the mean). The measured δ^{18}O and δ^{13}C values were not calibrated to the standard values (the Vienna Pee Dee Belemnite (VPDB) and the Vienna Standard Mean Ocean Water (VSMOW)) in order to exhibit the repeatability (standard errors and outliers) of the measurements. The curve and the equation indicate the relationship between the stable isotope value (*y*) and the pyrolysis temperature (*x*).

3.2 Calibration of the simultaneous measurement of δ^{13}C and δ^{18}O

We compared the results using different calibrated methods with data from simultaneous measurement (Fig. 2). The δ^{13}C from simultaneous measurement showed a clear decreasing trend in the two-point and multi-standard calibrations (Fig. 2a). Specifically, the multi-standard calibrated δ^{13}C decreased at an annual rate of –0.057‰, whereas the two-point calibrated δ^{13}C decreased at an annual rate of –0.041‰. The standard deviation of the multi-standard calibrated δ^{13}C (1.05‰) was higher than that of the two-point calibrated δ^{13}C (0.81‰) for the period 1950–2008 (Fig. 2a). There were some differences between the δ^{13}C values from the multi-standard calibration and from the combustion method, especially in the late 20th century, which resulted from the significantly depleted δ^{13}C in atmospheric CO_2 at this time period

(Woodley et al., 2012). The multi-standard calibrated δ^{13}C from simultaneous measurement had an offset (mean offset 0.43‰) with the δ^{13}C from the combustion method, and the offset was large at the end of the 20th century (Fig. 2a). This result is similar to the findings from other studies (Knöller et al., 2005; Leuenberger and Filot, 2007), which reported an offset between the tree-ring δ^{13}C results measured by the combustion method and the high-temperature pyrolysis method (at 1400°C). The multi-standard calibrated δ^{13}C from simultaneous measurement slightly underestimated the low δ^{13}C values obtained from the combustion method (Figs. 2a and b). These results differ from previous findings with "low-temperature pyrolysis" conducted at a temperature of 1090°C (Young et al., 2011). Specifically, the multi-standard calibrated δ^{13}C from simultaneous measurement slightly overestimated the low δ^{13}C values and underestimated the high δ^{13}C values, due to the potential influence of the glassy carbon.

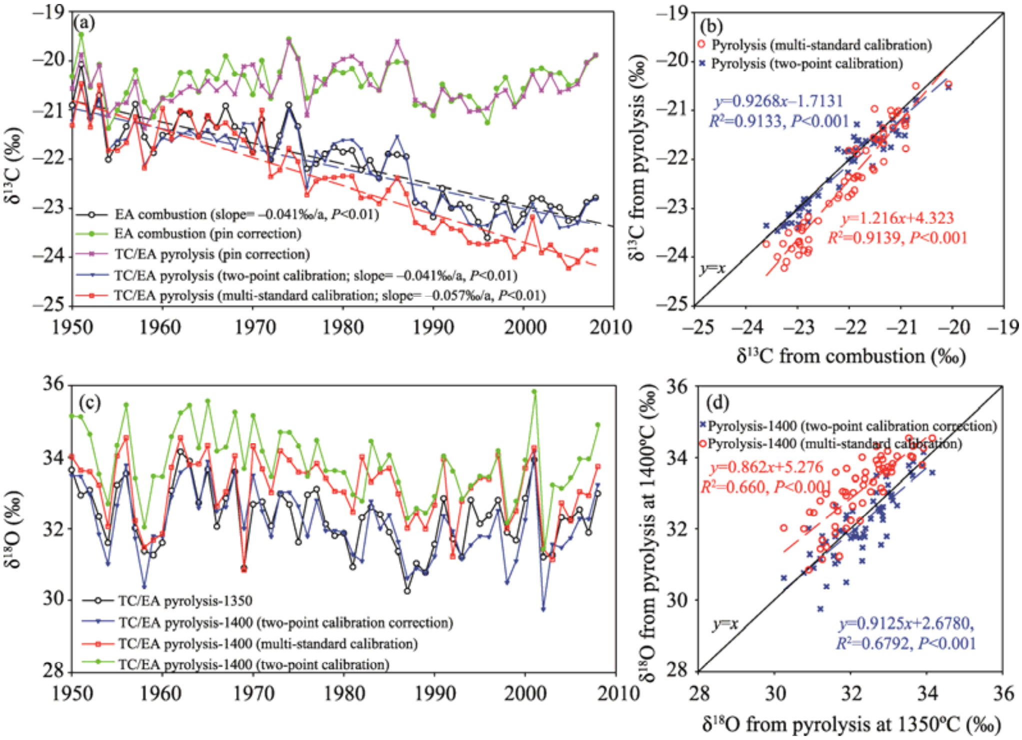

Fig. 2 (a) Time-series comparison of δ^{13}C values during 1950–2008 from 59 tree-ring α-cellulose samples combusted to CO_2 in an elemental analyzer Flash EA at 1050°C and trends of δ^{13}C values from two calibrated methods (multi-standard calibration and two-point calibration; Evans et al., 2016) for the pyrolysis to CO in the TC/EA at 1400°C. The "pin" corrected tree-ring δ^{13}C values from the combustion method and the two-point calibrated pyrolysis method were also exhibited. (b) Slopes and linear regressions for the δ^{13}C values from the combustion method and from the pyrolysis method calibrated by the multi-standard and two-point corrections. (c) Time-series comparison of tree-ring δ^{18}O values from 59 α-cellulose samples from the pyrolysis to CO in the TC/EA at 1350°C (pyrolysis-1350) and from the pyrolysis method (simultaneous measurement) at 1400°C (pyrolysis-1400). The two-point calibrated correction represents that the δ^{18}O values from the pyrolysis method (simultaneous measurement) at 1400°C (pyrolysis-1400) corrected the offset using the equations from Figure 1a (see the text for details). (d) Slopes and linear regressions for the δ^{18}O values from the pyrolysis method at 1350°C and the multi-standard calibrated δ^{18}O values and two-point calibrated δ^{18}O values after corrected offset from the pyrolysis method (simultaneous measurement) at 1400°C. For figures in the right pane, the black diagonal line represents the 1:1 line ($y=x$) and the dashed line represents the regression line.

The two-point calibrated δ^{13}C values from the pyrolysis method and the combustion method were very similar. Both δ^{13}C series showed a decreasing trend with an annual rate of –0.041‰, and there was no obvious offset (mean of 0.100‰) between them (Fig. 2a). After the "pin" correction of these two series, the $\delta^{13}C_{pin}$ values showed no significant trend from 1950 to 2008.

The δ^{13}C value from the combustion method was consistent with the value from the pyrolysis method for both the two-point and multi-standard calibrations (R^2>0.91; Fig. 2b), and both regressions were close to the 1:1 line. Our findings were better than the results reported by Woodley et al. (2012), who found large differences between the δ^{13}C values from the combustion and pyrolysis methods after the 20th century. These large differences could have been due to potential errors in methods, such as preferential deposition of carbon atoms, exchange with the glassy carbon, and formation of carbon residues in the carbon tube because of some uncompleted pyrolysis (Young et al., 2011). However, after we adopted the two-point calibrated method with two celluloses materials as standards, these errors were eliminated greatly because the two-point calibrated δ^{13}C had a smaller standard deviation and the slope of δ^{13}C from the two-point calibrated method and combustion method was closer to 1 (Fig. 2b). Our results also indicate that the two-point calibrated method of Evans et al. (2016) improves the precision of the δ^{13}C from simultaneous measurement (high-temperature pyrolysis) of the two isotopes compared with the multi-standard calibrated method.

There was an offset between the tree-ring δ^{18}O from the pyrolysis method at 1350°C and from simultaneous measurement at 1400°C (Figs. 2c and d). The offset was nearly constant (at about –1.52‰) for the two-point calibrated δ^{18}O during 1950–2008, but varied for the multi-standard calibrated δ^{18}O during some periods (e.g., 1970–1990). The years of 1970–1990 were a relative wet period (Xu et al., 2014), and the large variation of δ^{18}O in this period may result in a large offset variation. The δ^{18}O values from simultaneous measurement were higher than those from the pyrolysis method at 1350°C, which may result from the increased reaction temperature (Fig. 1). Because of the difference in δ^{18}O from the pyrolysis method at different temperatures, we applied an additional correction to the two-point calibrated δ^{18}O from simultaneous measurement by subtracting the difference (1.67‰) between the δ^{18}O values at 1400°C and 1350°C, which was calculated by the equations in Figure 1a. The offset-corrected δ^{18}O showed a very similar variation to the δ^{18}O from the pyrolysis method at 1350°C, and both of the two δ^{18}O series showed no significant temporal trends (Figs. 2c and d). In addition, the δ^{18}O from the pyrolysis method at 1350°C and from simultaneous measurement at 1400°C exhibited nearly parallel slopes (0.8620 and 0.9125 for the multi-standard and two-point calibrations, respectively), both of which were close to the 1:1 line, indicating that simultaneous measurement will not change the variability of the δ^{18}O from the pyrolysis method at 1350°C (Fig. 2d).

3.3 ANOVA test of the simultaneous and separate measurements

The mean δ^{13}C values for the *P. schrenkiana* samples based on the combustion and two-point calibrated simultaneous measurements were very similar (Fig. 3a) and did not differ significantly (–22.05‰ (±0.05‰) for the combustion method and –22.15‰ (±0.10‰) for simultaneous measurement; ANOVA test, P=0.513). The standard deviations of the 59 tree-ring α-cellulose samples were also similar (0.83‰ and 0.81‰, respectively, for the combustion method and simultaneous measurement). The range (maximum minus minimum) of the δ^{13}C from the combustion method was slightly larger than that from simultaneous measurement. The differences between the δ^{13}C values from the combustion method and simultaneous measurement were normally distributed (Shapiro-Wilk test, P=0.35), with most of the values in the range from –0.3‰ to 0.4‰ (Fig. 3b).

The mean δ^{18}O values for the 59 tree-ring α-cellulose samples from the separate pyrolysis method at 1350°C and from the temperature-corrected simultaneous measurement at 1400°C were also very similar and did not differ significantly (–32.31‰ (±0.25‰) for pyrolysis at 1350°C and –32.16‰ (±0.25‰) for simultaneous measurement at 1400°C; ANOVA test, P=0.386; Fig. 3c). The standard deviations were also similar, with the values of 0.87‰ and 0.97‰ for pyrolysis at 1350°C and for simultaneous measurement at 1400°C, respectively. The differences between the two methods were normally distributed (Shapiro-Wilk test, P=0.30), and most of the differences ranged from –0.5‰ to 0.5‰ (Fig. 3d). The range of the δ^{18}O values from simultaneous measurement was larger than that from the pyrolysis method at 1350°C (Fig. 3d). This means that after the correction of the mean δ^{18}O difference caused by the pyrolysis temperature, the means and standard deviations of δ^{18}O from simultaneous isotopic measurement were similar with the values obtained from separate isotopic analyses.

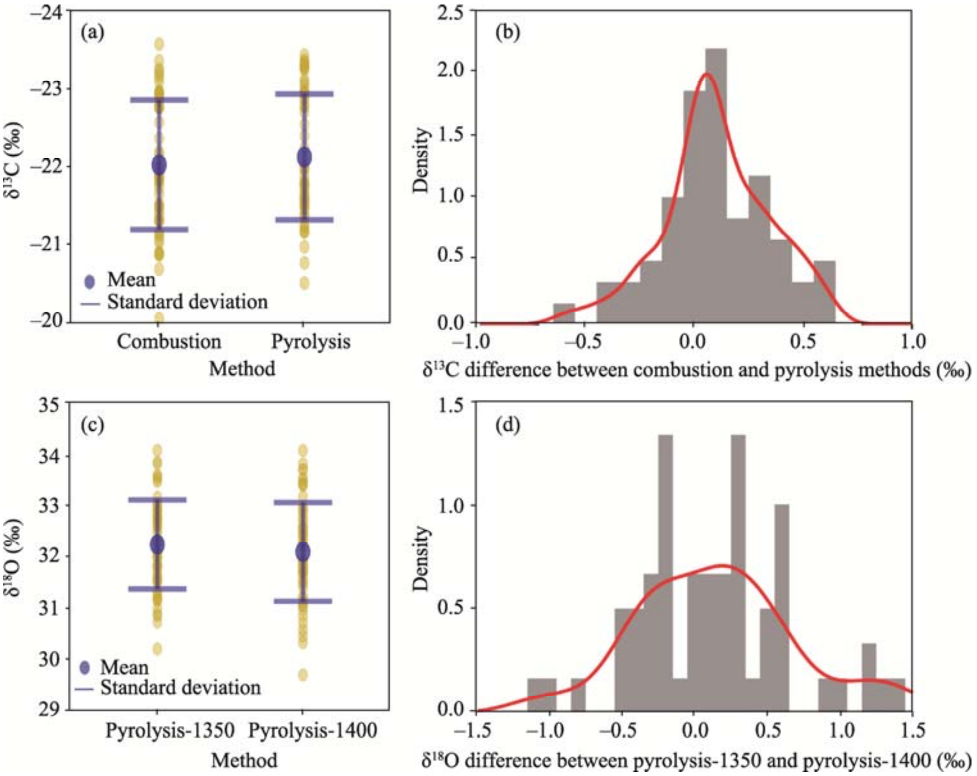

Fig. 3 (a) Comparison of δ^{13}C values from 59 tree-ring α-cellulose samples based on combustion and pyrolysis methods; (b) the density (width=0.1) distribution and density distribution curve (red line) of the differences between paired annual tree-ring δ^{13}C values determined using the combustion and pyrolysis methods; (c) comparison of δ^{18}O values from 59 tree-ring α-cellulose samples using the pyrolysis method at 1350°C (pyrolysis-1350) and the pyrolysis method (simultaneous measurement) at 1400°C (pyrolysis-1400); (d) the density (width=0.1) distribution and density distribution curve (red line) of the differences between paired annual tree-ring δ^{18}O values from the pyrolysis method at 1350°C (pyrolysis-1350) and the pyrolysis method (simultaneous measurement) at 1400°C (pyrolysis-1400). In the left pane, values are means (purple dots)±standard deviations (error bars) for 59 tree-ring α-cellulose samples (yellow dots). Values in the left pane did not differ significantly (ANOVA-test) between the two methods.

The simultaneously measured δ^{13}C and δ^{18}O values (pyrolysis method) were typically similar to the separately measured values (combustion and pyrolysis methods, respectively), with non-significant differences between them. Simultaneous measurement may be considered less accurate than separate measurement, especially for the δ^{13}C determined by the combustion method. Young et al. (2011) suggested that if suitable laboratory procedures (such as those described in their paper) were adopted, any small loss in accuracy would be outweighed by the greater efficiency of simultaneous measurement of two isotopes (i.e., the potential to greatly decrease the sample size). For tree rings, the variability between samples from different individuals (1‰–3‰ for δ^{13}C and 1‰–4‰ for δ^{18}O; Leavitt, 2010) was much greater than the analytical uncertainty and the isotopic offset in different measurement methods (–0.3‰–0.4‰ for δ^{13}C and –1.0‰–1.5‰ for δ^{18}O; Fig. 3), and even for trees growing in close proximity, the difference between individuals can be larger than the difference in the records of climate change within an individual tree (McCarroll and Loader, 2004; Leavitt, 2010). Although our research was conducted in an arid area, the results are consistent with findings from humid areas (Young et al., 2011; Woodley et al., 2012). That is, the $\delta^{13}C$ and $\delta^{18}O$ values from simultaneous measurement are similar to those from separate measurement. This indicates that the simultaneously measured $\delta^{13}C$ and $\delta^{18}O$ can be applied both in humid and arid areas. In climate reconstructions, the precision of the estimated isotopic average values in tree rings depends much more on the number of trees in the sampling than on the precision of individual measurements of the isotope values (McCarroll and Loader, 2004; Young et al., 2011). Therefore, the simultaneous measurement could produce an acceptable regional isotope chronology due to the small decrease of accuracy, combined with the similar mean values and trends for tree-ring $\delta^{13}C$ and $\delta^{18}O$ (Figs. 2 and 3).

3.4 Climate response of tree-ring $\delta^{13}C$ and $\delta^{18}O$

The relationships between the stable isotopic values ($\delta^{13}C$ and $\delta^{18}O$) and the four climate variables (mean monthly temperature, total monthly precipitation, mean monthly relative humidity and SPEI) were very similar, regardless of whether the isotopic values were from separate measurement or from simultaneous measurement (Fig. 4). The climate response inferred from the combustion $\delta^{13}C_{pin}$ was significantly ($P<0.01$) positively correlated with mean temperature in July ($r=0.33$), and negatively correlated with relative humidity in July, SPEI in July, and SPEI of the whole growing season from May to August($r= -0.38$, $P<0.01$; Fig. 4). The climate response results for the $\delta^{13}C_{pin}$ from the pyrolysis method suggest that $\delta^{13}C$ was significantly positively correlated with the overall mean temperature of the whole growing season ($r=0.40$, $P<0.01$), and was significantly ($P<0.01$) negatively correlated with precipitation and SPEI during the whole growing season (Fig. 4). The $\delta^{13}C_{pin}$ from the pyrolysis method did not alter the pattern of $\delta^{13}C$-climate response and kept the climatic signals. Furthermore, it reveals that some significant climatic signals were not detected by the combustion method (e.g., negative and significant ($P<0.01$) correlations for temperature in December of the previous year, and for SPEI in May of the current year; Fig. 4). The $\delta^{13}C_{pin}$ from the pyrolysis method enhanced the climatic signal strength of overall mean temperature, precipitation, relative humidity, and SPEI for the whole growing season (Fig. 4). These results indicate that the $\delta^{13}C_{pin}$ from the pyrolysis method does not greatly alter the interpretation of the climate response of tree-ring $\delta^{13}C$.

Tree-ring $\delta^{18}O$ values from the pyrolysis method at 1350°C and from simultaneous measurement at 1400°C were significantly ($P<0.01$) positively correlated with mean monthly temperature from May to August ($r>0.36$) and the overall average temperature of the whole growing season ($r=0.54$). However, they were significantly ($P<0.01$) negatively correlated with precipitation in July, relative humidity from May to July, and SPEI in May and July (Fig. 4). Both of the two tree-ring $\delta^{18}O$ series (from the pyrolysis method at 1350°C and from simultaneous measurement at 1400°C) showed significant correlations ($P<0.01$) with the overall mean temperature ($r=0.54$ and $r=0.53$, respectively), precipitation ($r= -0.42$ and $r= -0.44$, respectively), relative humidity ($r= -0.49$ and $r= -0.49$, respectively) and SPEI ($r= -0.51$ and $r= -0.54$, respectively) for the whole growing season (Fig. 4). The tree-ring $\delta^{18}O$ from simultaneous measurement, therefore, did not alter the dominant climatic signals in the tree-ring $\delta^{18}O$ series, and clearly reflected both temperature and moisture conditions during the growing season (Xu et al., 2014). As was the case for $\delta^{13}C_{pin}$, the $\delta^{18}O$ from simultaneous measurement reveals that some significant ($P<0.01$) climatic signals were not detected in separate measurement (e.g., precipitation in April of the current year; Fig. 4).

Because the climate response analyses or climate reconstruction would re-scale the series of tree-ring stable isotopes, the climatic signals from the pyrolysis and combustion methods would change little (Young et al., 2011). Taken together, our results indicate that the climate response of tree-ring $\delta^{13}C$ and $\delta^{18}O$ obtained from simultaneous measurement is the same as that from separate measurement, and therefore simultaneous measurement can be used as an alternative method for detecting the sable isotopic values ($\delta^{13}C$ and $\delta^{18}O$). These good results can be achieved due to the low loss of accuracy and the similar regional mean values and trends provided by the simultaneous measurement (Figs. 2 and 3). Thus, for analysis of a regional tree-ring isotope network, the simultaneous measurement would greatly improve the number of sampling points that can be analyzed, and will therefore improve the regional coverage.

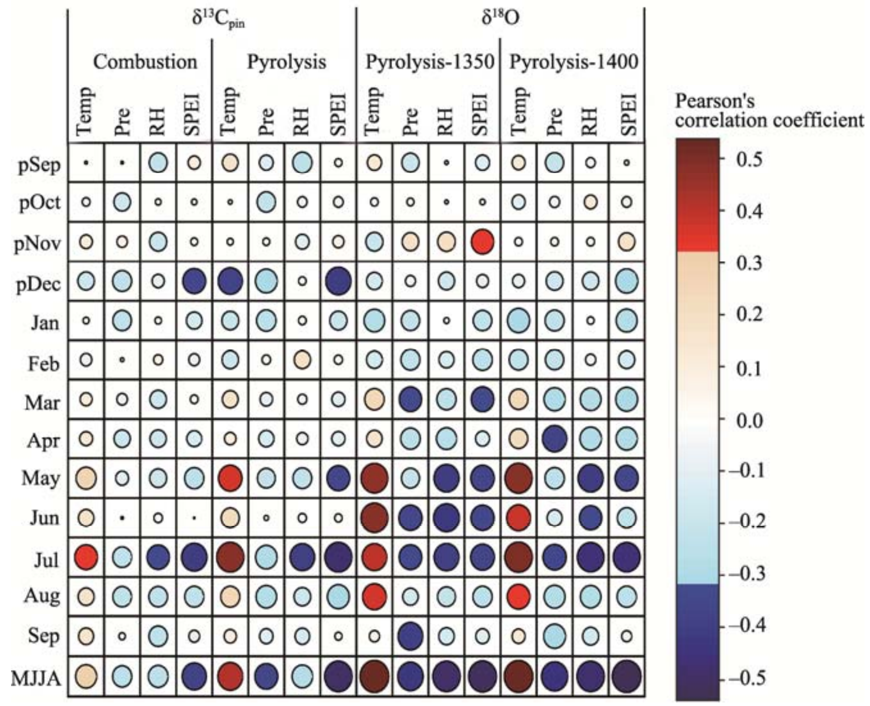

Fig. 4 Climate responses of tree-ring $\delta^{13}C_{pin}$ and $\delta^{18}O$ series determined from separate and simultaneous measurements. For simultaneous measurement, the stable isotope values were calibrated with the two-point method (Evans et al., 2016). The tree-ring $\delta^{13}C_{pin}$ was the $\delta^{13}C$ corrected by using the method of "pin" correction (McCarroll et al., 2009). The size and color of the circles indicate the strength and significance of the relationship (Pearson's correlation coefficient), respectively. The red and blue circles indicate the Pearson's correlations are statistically significant ($P<0.01$). Months with a "p" prefix represent months in the previous year. MJJA represents the mean value from May to August in the growing season of the current year. Temp, mean monthly temperature; Pre, total monthly precipitation; RH, mean monthly relative humidity; SPEI, monthly mean standardized precipitation-evapotranspiration index.

4 Conclusions

In this study, we compared the $\delta^{13}C$ and $\delta^{18}O$ values obtained from separate and simultaneous measurements to determine the suitability of simultaneous measurement using the high-temperature pyrolysis method in an arid region of northwest China. We found that the $\delta^{13}C$ values from the combustion method were very similar to those from simultaneous measurement (high-temperature pyrolysis method). The two-point calibration method improved the results for both $\delta^{13}C$ and $\delta^{18}O$, and the mean value, standard deviation, and trend of the calibrated $\delta^{13}C$ series from the pyrolysis method (simultaneous measurement) were similar to those obtained from the combustion method (separate measurement). The simultaneously measured $\delta^{18}O$ values from the pyrolysis method at 1400°C showed a nearly constant offset compared with the values from the pyrolysis method at 1350°C because $\delta^{18}O$ depends on the reaction temperature. However, the variability of the $\delta^{18}O$ from simultaneous measurement was similar to that from separate measurement in the time series. The correlation of the stable isotope values with temperature and moisture variables indicates that simultaneous measurement will not change the inferred climatic signals. Based on the results mentioned above, we conclude that simultaneous measurement of the $\delta^{13}C$ and $\delta^{18}O$ values in tree-ring α-cellulose samples through high-temperature pyrolysis method would produce acceptable and reliable stable isotope series in both arid and humid areas. The simultaneous isotopic measurement can greatly reduce the cost and time requirement compared with separate isotopic measurement.

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